Letter to the Editor

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Practical approach for medical validation of therapeutic drug monitoring results

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To the Editor,

Validation of results is one of the core tasks of laboratory medicine specialists. This work includes a technical component, often achieved by maintaining internal and external quality control (QC) within preset limits, but also has an important medical component. By reviewing results, a specialist can filter out unexpected or contradictory results and subject them to further examination. The specialist hereby has to integrate knowledge of pathophysiology with technical competence. This manual test validation has the disadvantage of being time-consuming with large inter-individual variation which subsequently slows down the response time of the laboratory to the clinic. Specialists try to cope with this problem by installing pre-defined triggers for absurd values (probably caused by a technical or sampling flaw, panic values, etc.) and by reviewing of a subset of 'unexpected' results, where unexpected is often defined as an abnormally high or low value or, most often, as a consecutive value trespassing the reference change value (RCV) based on within-subject biological variation and analytical CV (delta check). A possible solution can be found in commercial packages that are extensively used to filter out results for review [1, 2]. To efficiently use such models, there is a need for useful estimates of biological variation. For therapeutic drug monitoring (TDM) this has been historically a difficult issue. Recently, we published practice-oriented criteria for some drugs based on a proxy for biological variation of drugs in blood both within-and between-subject [3]. Here we present similar calculations for a large number of therapeutic drugs monitored in our lab. Further, we use the calculated within-subject variations in a delta check algorithm to filter out results which could merit closer inspection. We give proof-of-concept for vancomycin and tacrolimus, representing the two drug classes (i.e. antibiotics and immunosuppressants).

For five antibiotics and five immunosuppressants routinely monitored in our lab, we retrospectively checked our laboratory information system during a 1-year period (December 1st, 2012 to November 31st, 2013). We included consecutive drug level determinations for patients receiving the medication that were both within the proposed therapeutic range to exclude possible dosing changes, pre-analytical flaws or major patient instabilities. We further calculated the difference between the two consecutive concentrations for each patient and drug. Outliers in these differences, unlikely to be caused only by biological variation, were detected and excluded by means of a Tukey filter [4]. Determinations were made by the same method on the same instrument. Everolimus, sirolimus and mycophenolate are measured using liquid chromatography mass spectrometry (LC-MS/MS) using an in-house validated assay [5, 6]; tacrolimus and cyclosporine are measured using the Architect SR2000i immunoassay. Vancomycin, gentamycin, amikacin and tobramycin concentrations are determined on a Roche Cobas 8000 (Roche Diagnostics, Mannheim, Germany). For voriconazole, a previously described LC-MS/MS assay is used [7]. Within- and between-patient variability was calculated

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using the CLSI EP5A-02 standard protocols [8]. Further, the analytical variability (CV_A), which was determined by the monthly instrument CV on the quality control level closest to the average of the respective consecutive measurements, was subtracted from the raw within-subject ($CV_{I,raw}$) or between-subject ($CV_{G,raw}$) variation using the formula $(CV^2_{I/G,raw})-(CV^2_A)=(CV^2_{I/G})$. For both immunosuppressants and antibiotics, the results are presented in Table 1. We proposed quality specifications based on the feasibility index (TEa/CV_A).

Further, we prospectively examined the differences between consecutive measurements for two patients receiving vancomycin and tacrolimus, respectively. To assess the significance of the differences of serial results, the 95% RCV was calculated using the formula: $2^{1/2}z(CV^2_A-CV^2_I)^{1/2}$; were z is the coverage factor (1.96) corresponding to the 95% confidence interval, CV_A the analytical coefficient of variation and CV_I the corrected within-subject variation [9]. For each sample, the requesting physician was contacted to ask the dose of the drug the patient received, the time at which the drug was administered, and the time at which a sample for TDM was drawn. The results are presented in Figure 1.

Based on this RCV filter, we propose an algorithm to identify samples that merit closer inspection. We suggest that all first patient values outside the therapeutic interval are reviewed by a laboratory medicine specialist giving a comment on how to change the dose and when to re-evaluate the effect. For values within the therapeutic interval, no review is needed and the RCV can be used for serial results to check for major pre-analytical flaws, unnecessary dosing changes or unstable patient situations. For values outside the reference interval, the calculated RCV on the serial result can be used to calculate whether the dosage change had an effect (if there was one). If the RCV is not surpassed or if the serial value does not enter the therapeutic interval, special attention from the laboratory medicine specialist can be of value. By this algorithm, only 30% and 22% of the samples of our vancomycin and tacrolimus patient would need attention, respectively.

We aimed to determine the normal between-person variation in a steady-state therapeutic situation (as a proxy for healthy individuals in a biological variation study for endogenous analytes), after the therapeutic regimen has been optimized for the patients specific characteristics (e.g. genetics). Thus our study design does not cover the total between-patient variation of drug levels. We are aware that taking these factors into account the total variation would be higher. In fact, these factors are the reason for performing TDM of these drugs.

Table 1: Quality specifications for optimal, desirable or minimal imprecision (I), bias (B) and total error allowable (TEa).

Analyte	n	CV_A	Interpretative comment or therapeutic interval	Mean concentration (SD)		Median time interval (IQR)	Observed variation		Quality specifications			TEa/CV_A	Quality level	95% RCV
				First measurement	Second measurement		CV_I, %	CV_G, %	I, %	B, %	TEa, %			
Everolimus	70	6.4	Therapeutic: 3.0–8.0 µg/L	4.9 (1.2)	4.8 (1.1)	34 days (12–83)	10.6	19.2	8.0	8.2	21.4	3.3	Minimal	34.4
Cyclosporin	234	12.3	Toxic trough >350 µg/L	117.5 (53.9)	122.5 (54.4)	28 days (2–87)	3.5	41.5	NA	NA	NA	1.6[a]	Analytical	35.4
Mycophenolate	354	7.9	Therapeutic: 1.0–3.0 mg/L	1.7 (0.5)	1.7 (0.5)	30 days (9–77)	4.5	27.5	NA	NA	NA	2.0[a]	Analytical	25.2
Sirolimus	46	6.9	Therapeutic: 4.0–20.0 µg/L	8.5 (3.2)	8.4 (3.1)	34 days (6–73)	18.7	30.8	9.3	9.0	24.4	3.5	Desirable	55.2
Tacrolimus	1603	3.9	Therapeutic: 5.0–15.0 µg/L	8.6 (2.3)	8.6 (2.3)	35 days (1–91)	6.2	25.2	3.1	6.5	11.6	3.0	Desirable	20.3
Amikacin	130	5.5	Toxic trough >5.0 mg/L	2.3 (1.1)	2.1 (0.9)	5 days (1–10)	22.7	41.3	11.4	11.8	30.6	5.6	Desirable	64.9
Gentamycin	40	3.0	Toxic trough >2.0 mg/L	0.8 (0.4)	0.8 (0.4)	1 day (1–2)	14.5	50.2	3.6	6.5	12.5	4.2	Optimal	41.0
Tobramycin	36	3.2	Toxic trough >3.0 mg/L	0.6 (0.3)	0.7 (0.3)	1 day (1–3)	23.8	45.3	5.9	6.4	16.2	5.1	Optimal	66.5
Vancomycin	700	4.5	Therapeutic: 10.0–20.0 mg/L	14.3 (2.6)	14.3 (2.6)	1 day (1–2)	8.9	14.3	6.7	6.3	17.4	3.9	Minimal	27.7
Voriconazole	40	6.1	Therapeutic: 2.0–5.5 mg/L	3.5 (1.0)	3.5 (0.8)	4 days (2–7)	12.9	20.8	9.7	9.2	25.2	4.1	Minimal	39.6

Observed within-subject (CV_I), and between-subject variation (CV_G), corrected for analytical variation for immunosuppressant's and antibiotics. [a]For cyclosporine and mycophenolate, a feasibility index <3 was obtained for the minimal quality specifications. Therefore, no quality specifications are defined. NA, not applicable.

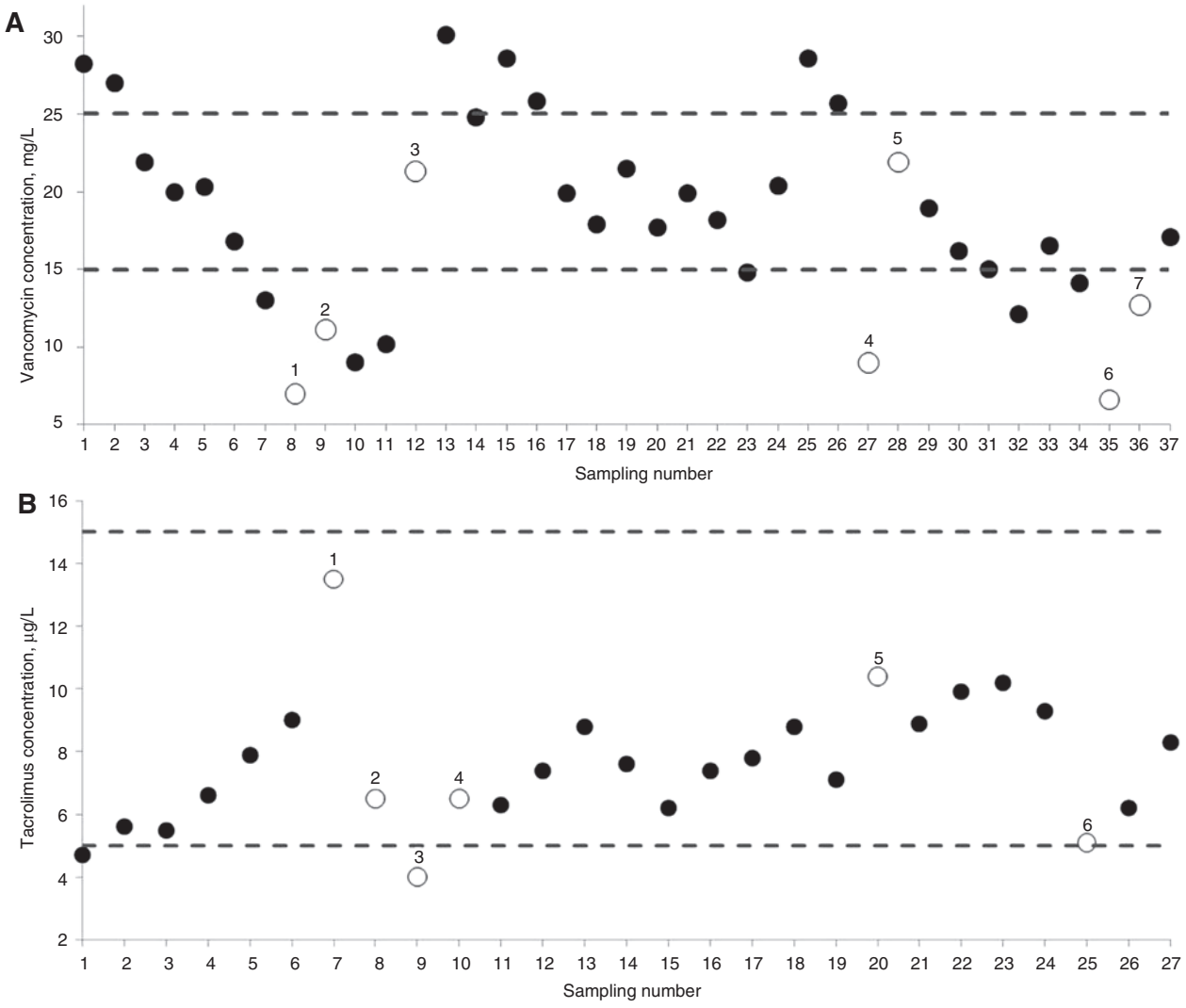

Figure 1: Vancomycin concentration profile (A) and tacrolimus concentration profile (B) of two different patients.
The open dots present the concentrations that fall out of the 95% reference change value (RCV). For each profile, a reason for the difference is given. Panel A: 1. Concentration 7.0 mg/L: patient did not receive vancomycin; 2. 7.0 mg/L: patient did not receive vancomycin; 3. Concentration 21.3 mg/L: change in vancomycin dose; 4. Concentration: 9.0 mg/L: patient did not receive vancomycin; 5. Concentration: 21.9 mg/L: restart of vancomycin dose; 6. Concentration 6.6 mg/L: wrong sampling time (20 h instead of 14 h); 7. Concentration: 12.7 mg/L: restart vancomycin dose. Panel B: 1. 13.5 µg/L: patient received a double dose (8 mg instead of 4 mg); 2. 6.5 µg/L: change in tacrolimus dose; 3. 4.0 µg/L: patient did not receive tacrolimus; 4. 6.5 µg/L: restart tacrolimus administration; 5.: 10.4 µg/L: patient received 7 mg instead of 4 mg; 6. 5.1 µg/L: patient did not receive tacrolimus.

We also noticed that for some drugs, the calculated within-subject variation was lower than the currently achieved analytical variation (e.g. cyclosporine, mycophenolate) (Table 1). This implies that the lion's share of variability between two consecutive results within the therapeutic range can be ascribed to analytical variation. The implication is that true but smaller changes in steady-state concentrations will be swamped by analytical variations, but larger and clinically relevant sampling or dosing errors are picked up. We are aware that the calculated biological variations can still be contaminated with some pre-analytical variation. Also, deviations in dose or health from patients cannot be excluded. However, we introduced some precautions by using only consecutive levels within the therapeutic range and also an outlier filter for unusually large differences between consecutive measurements, indicating deviations or substantial pre-analytical flaws. Last, we provided proof-of-concept for the applicability of the calculated biological variation to filter out relevant results (dosing change, pre-analytical flaws) in our setting.

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